Week 3 Notebook

June 15, 2015 - June 19, 2015

June 15, 2015

Kayla/Julie

- Analyzed sequencing results for 15-15C
    - identified alignments in forward and reverse directions
        - Forward sequence

NNNNNNNNNNNNNNNNNNNACNNNNNNNAATAGGCGTATCACGAGGCAGAATTTCNGATAAAAAAAATCCTTAGCTTTCGCTAAG
GNNGNNTTCTGGAATTCGCGGCCGCTTCTAGAGAAAGAGGACAAATACTAGATGCGTAAAGGAGAAGAACTTTTCACTGGAGTT
GTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATAC
GGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCGGTTATGGTGT
TCAATGCTTTGCGAGATACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAG
AACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATANAATCGAG
TTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCAT
GGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTANACACAACATTGAAGATGGAAGCGTTCAACTANCAGACCAT
TATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAAACAACCATTACCTGTCCACACAATCTGCCCTTTCNAAAG
ATCCCAACNAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTNACAGCNTGCTGGGATTACACATGGCATGNATGANCTATAC
AAATAATAATACTANAGCCAGGCATCCAATAAAAACGAAAGGCTCANNNAAAAAACTGGGCCTTTCNTTTANNCTGTTGTTTNGT
CGNNGAACGCTCTCTACNANNTCACACTGGCTCACCNTCNGGTGGGNCTTTCNNNNTTTATAATACTAGTANCGGCCGCTGCA
NTCCGGAAAAAANNCAAGGTGTCACCCCCNTGCCCTTTTTNNNTTAAANCGAAAANAATNACTTCNCGTNTNN

        - Reverse Sequence

NNNANNNGATNTNTTNNNNNNGNNNNAANNNNNTNTNNNGNNNNCGANNCANTNNNNNNCNCCNGNNGTNNNGAANCNTNNT
NNNCNNNNCNTNNCNNNAAANNNGNNNNCNNNNNGGCAGANTTCNGNNAAAAAAAAANNNAGCTTTCGNTAAGGATGNTTNNGA
ATTCGNNGNCGCTCTAGAGAAAGNGNGAAATNCTAGATGCGTAAAGNAGAAGAACTTTCACTGGAGTNGTCCCAATTCTTGTTG
AATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCNTA
AATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGA
TACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAG
ATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTT
TAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAG
AATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTC
CAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGA
GAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAATAATACTAGAG
CCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTA
GAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATACTAGTAGCGGCCGCTGCAGTCCGGCAAAAAAGGGCAA
GGTGTCACCACCCTGCCCTTTTTCTTTAAAACCGAAAAGATTACTTCGCGTTATGCAGGCTTCCTCGCTCACTGACTCGCTGCG
CTNNNTNNGTNNNNNNNNNNNNNNNNNN

- 5 mL liquid cultures of 23716 and 15224 and a 50 mL culture of DH5α in LB

Charlotte/Chloe

We began preparing our presentation for the NEGEM meeting this week. At the end of the day, we prepared cultures of 14-34C, 14-35C, RFP from transformation test kit (all for a repeat of the freezing survival assay), and the bright GFP + J23108 colony (to make a miniprep). Some graphics for both the website and the powerpoint presentation were created.

Dave/Eddie

- Started minipreps of four colonies each picked from plates of 15-11C and 15-18C and left them overnight.
- We made 2L LB broth and 2L CAM plates
- We worked on the NEGEM presentation
- Ran a PCR of the 15-4C ZeAFP in a 50 ul reaction with the following ingredients:
    - 1.3 ul each biobriock forward and reverse primers, diluted to 1:10
    - 25 ul 2x PCR reaction solution
    - 1 ul 15-4C
    - qs to 50 ul with H2O (11.5 ul)
- and with the following program (titled iGEM PCR) in the left-most PCR machine
    -

Initial Denaturation	95C	1 min
Cycle 32x	95C	30 s
	55C	30 s
	72C	30 s
Final Extension	72C	2 min

- we then ran the product in a gel to confirm length
    - The first well was skipped due to an air bubble in the well
    - The third well was redone in the fourth well as the gel was pierced by the pipette tip
    - 1 ul of PCR product was run

June 16, 2015

Kayla/Julie

- Prepared biofilm assay using liquid cultures prepared yesterday
    - 20 uL of cells into 1.98 mL of LB Media, Difco Minimal Media, and M9+L-Arginine Minimal Media
    - 1 96 well plate at 37C for 48 hours
        - A1-12 and F1-12=DH5α
            - 1-4=LB
            - 5-8=Difco Minimal Media
            - 9-12=M9+L-Arginine
        - B-12 and G1-12=23716
            - 1-4=LB
            - 5-8=Difco Minimal Media
            - 9-12=M9+L-Arginine
        - C1-12 and H1-12=15224
            - 1-4=LB

- 5-8=Difco Minimal Media
- 9-12=M9+L-Arginine

Charlotte/Chloe

The cultures of 14-34C, 14-35C, and the RFP from the transformation kit were diluted per the previously set freeze assay protocol. In this case, only the 10^{-6} and 10^{-7} dilutions were plated, and only the stationary phase bacteria were used. 0.5 mL aliquots of all three cultures were placed at both -20℃ and -80℃ and all plated dilutions were left to incubate overnight at 37℃

A miniprep of the glowing colony suspected to be J23108+GFP Insert was prepared. The final concentration of DNA was calculated to be 615 ug/ml.

A test digest of the glowing colony was prepared by Dave & Eddie and a gel was run.

Sequencing for the glowing colony (15-11C), a 15-11C sample prepared by Dave and Eddie, and a 15-18C miniprep was prepared and left to be picked up at 3 pm.

Dave/Eddie

- Continued the minprep from yesterday on four samples each of 15-11C and 15-18C.
- Found the concentrations of each sample:

	Absorbance @260	Absorbance @280	Concentration
15-11C 1	0.0003	0.0003	
15-11C 2	-0.0014	-0.0014	
15-11C 3	0.0021	0.0007	105
15-11C 4	0.0173	0.0100	865
15-18C 1			
15-18C 2			
15-18C 3			
15-18C 4			

*table left unfinished because spectrophotometer was acting up.

- We will submit the samples for gene sequencing, one from each of the four. This can be done only if the test gel confirms that the lengths are all the same and the expected size.
- 15-18C and 15-11C were digested with X and P then a test gel was run to confirm sizes, along with the GFP colony miniprep. 1 ul DNA and 0.5 ul dye were used.
    - 1.Ladder
    - 2.15-1C 1

    - 3.15-11C 2
    - 4.15-11C 3
    - 5.15-11C 4
    - 6.15-18C 1
    - 7.15-18C 2
    - 8.15-18C 3
    - 9.15-18C 4
    - 10. GPF colony
- The PCR product from yesterday 15-4C was run on a gel in 2 wells to check length before digesting with S and P
- The lowest band was cut and extracted. The extraction is according to protocol except that 30 ul H2O was used to elute rather than 50 ul elution buffer
- We ran a gel of the uncut vectors for 15-18C and 15-11C and the GFP. The gel showed that there was vector in each well, though not much in 15-18C wells. Part of the problem is that only 1 ul was run on the gel, in the future test digests should be run with half to all of the digest on the gel as the remainder is unnecessary.
- Ligations were done of 15-4C, using 2 ul 15-17C and 2, 5, or 12 ul of the insert, using both the old insert from 6/11/15 and the new PCR purified insert from today. A no insert control was also made.

June 17, 2015

Kayla/Julie

- Analyzed sequencing results for 15-11C and 15-18C
    - none of the sequences resembled the expected sequences
        - decided to repeat ligations for 15-18C and measure the DNA concentrations of 2 of the 15-11C minipreps before sending them off for sequencing again
            - GFP1=4 ng/uL
            - GFP2=325 ng/uL
- Performed a test transformation to compare efficiency of 23716 competent cells to NEB competent cells

Charlotte/Chloe

None of the sequencing worked. The most likely reason for this is inaccuracies in reading DNA concentration by our spectrophotometer. Select minipreps will be run to Gateway to be analyzed with the nanodrop and new forward and reverse primers have been ordered for the prefix and suffix.

Serial dilutions of the cultures left overnight at both -80℃ and -20℃ for the freeze survival test were prepared. Both the 10^{-6} and the 10^{-7} dilutions were plated for each sample, and all 12 plates were left to incubate overnight at 37℃.

The pre-freeze plates were taken out of the incubator at 11 am and the colonies for each plate were counted, as seen in the table below.

Colonies of Stationary Phase Growth *E. coli* Plates (Before Freeze)					
Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies
34C 10-6	374	35C 10-6	1067	RFP 10-6	611
34C 10-7	179	35C 10-7	180	RFP 10-7	71

PCRs of the glowing colony's miniprep (15-11C), a 15-18C miniprep, and two ZeAFP minipreps were prepared using the VF2 AND VR primers. Dave and Eddie prepared the same PCRs using the 5' prefix and 3' suffix primers, as well.

Dave/Eddie

- We will be performing seven transformations, of the ligations made yesterday.
- 2 ul of ligation were used for each transformation. Transformation conducted according to protocol.
- Sequence analysis came back, showing that we had no insert or that the sequencing was bad and had to be redone. We are redoing 15-7C ligated with 15-5A using the following reactions:

Code	Buffer	Ligase	Vector 15-7C	Insert 15-5A	H2O	Total
15-18C Con	2	1	3	0	14	20
15-18C 1	2	1	3	3	11	20
15-18C 2	2	1	1	2	14	20
15-18C 3	2	1	1	5	11	20
15-18C 4	2	1	1	9	7	20

- Ligations left at RT 1 hr.
- Ligations transformed using 2ul of ligand and 50 ul competent cells and left overnight
- We helped with PCR and gel test of two ZeAFP samples as well as the chloeGFP. Standard PCR as done earlier.
- Wells in the gel:
    - 1 ladder

    - 2 GFP VFVR
    - 3 GFP 5'3'
    - 4 111 VFVR
    - 5 111 5'3'
    - 6 Z1 VFVR
    - 7 Z1 5'3'
    - 8 Z2 VFVR
    - 9 Z2 5'3'
    - 10 GFP 5'3'
- Well 3 is repeated in well 10 because of a pipetting error which caused nothing to be loaded into well 3.

June 18, 2015

Kayla/Julie

- Analyzed sequencing results for 15-11C
    - priming failed
        - new VF2 and VR primers ordered
- Counted colonies on transformation efficiency plates
    - Our attempt at making competent cells was a success - the plates grew many colonies
- Stained biofilm assay with crystal violet
    - Biofilms formed for EMG2:K(lambda) (previously referred to as 223716) in M9 Minimal Media
- Digested 15-5A
    - Incomplete - gel showed only plasmid and no bands of just the insert

Charlotte/Chloe

Colonies were counted on post-freeze plates and the percent survival was calculated for each strain.

Colonies of Post-Freeze *E. coli* Plates						
Strain	Temp (°C)	Growth phase	Colonies from 10-6	Percent Survival	Colonies from 10-7	Percent Survival
35C	-20	stationary	162	15.18%	52	28.88%
34C	-20	stationary	216	57.75%	73	40.78%
RFP	-20	stationary	1	0.16%	4	5.63%
35C	-80	stationary	33	3.09%	9	5.00%

34C	-80	stationary	31	8.29%	5	2.79%
RFP	-80	stationary	0	0%	0	0%

Dave/Eddie

- No colonies on the 15-18C plates, though not totally unforeseen. As those parts are useless due to a missing promoter, we discarded the plates. The ZeAFP plates did not grow anything obvious, but were left for the end of the day to see if anything grows.
- The PCR product ZeAFPs were extracted using the gel extract kit. They were digested using X and P, then gel purified with the following wells:
    - 1 Ladder
    - 2 Z VFVR
    - 3 Z VFVR
    - 4 Z 5'3'
    - 5 Z 5'3'
- The ZeAFP was then extracted. Each extraction used 30 ul of water for the elution. The digest was conducted in 50 ul, using all 30 ul from the extraction, 2 ul each X and P, 5 ul buffer, and qs to 50 with H2O. The digest ran for 1 hr at 37C. We gel purified in the 1.5% gel, extracted and again eluted with 30 ul H2O, and then stored at -20C.

June 19, 2015

The whole team attended NEGEM 4 at Boston University and presented a small talk about the project as it is thus far.